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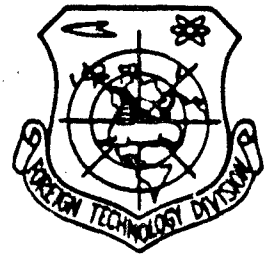
FOREIGN TECHNOLOGY DIVISION

BIOLOGICAL TISSUE SENSORS

by

Liu Jinhua, Luo Meiqin



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BIOLOGICAL TISSUE SENSORS

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ABSTRACT

This paper describes the structure of a tissue electrode and the optimal pH conditions, as well as the effect of factors such as glycerine and the diameter of the nylon net holes on tissue electrodes. It also undertakes a comparison between tissue electrodes and other varieties of biological electrodes. It presents an example of the practical application of tissue electrodes. From this example it can be seen that tissue electrodes have broad prospects for application in biomedicine.

The ion selection electrode has been one of the most rapidly developing analytic technologies since the end of the late sixties, and the biological catalytic membrane electrode is one area that has begun to develop in recent years. Because the biological membrane electrode has unique analytic functions and practical value in clinical chemistry, it has attracted serious attention. Fixing a layer of intact animal or plant tissue on the surface of an ionic selection primary electrode creates a tissue electrode, which, with a variety of substances in the enzyme catalyzed base fluid contained in the biological material, produces gases (like NH_3, O_2 or CO_2) sensed by the gas membrane electrode, allowing attainment of the goal of measuring of the base substance concentration. Development of the first tissue electrode began in 1978; it was a glutamine electrode [12] constructed by joining fresh pork kidney and NH_3 electrodes. Not long after that, by joining a pumpkin skin layer tissue section containing large quantities of glutamic acid carboxyl-removing enzyme with a CO_2 electrode, an L-glutamic acid plant tissue electrode [8] was constructed, following which still other animal and plant tissue electrodes made their appearance.

STRUCTURAL AND MEASUREMENT PRINCIPLES OF THE TISSUE ELECTRODE

Generally speaking, tissue electrodes are composed of animal or plant tissue and a permeable membrane forming a sandwiched layer, to which is joined a gas-sensitive electrode. Figure 1 is a diagram of a glutamine tissue electrode formed by joining a porcine kidney and an NH_3 electrode. It represents the structure of the ordinary tissue electrode. Section a in the figure, cut from a biological specimen, is separated above and below using an osmotic analysis membrane c and a monofilament nylon net cover, tightly affixed to the gas permeable membrane d. The gas (·) that is produced diffuses into the electrode internal electrolyte solution e, changing the internal electrolyte solution's pH value, and is monitored by the pH value electrode f. Because the quantity of gas produced has a certain function relation with the base fluid concentration, the goal of fixed quantity measurement is attained. The thickness of the tissue slice may range between several dozen millimeters and several hundred millimeters. On the basis of reports in the literature [12], when the size of the slice is the same as the size of the surface of the pH glass electrode, the electrode effect is ideal. Table 1 shows the tissue electrodes that have been reported to date, along with the corresponding material used in their construction.

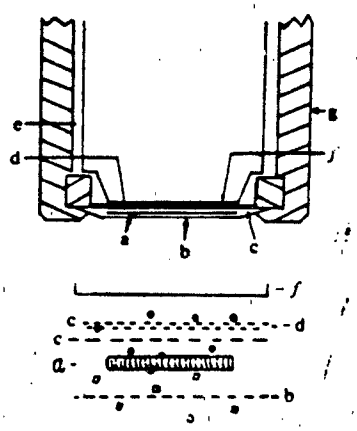

Fig. 1.

RESPONSIVE PROCESSES OCCURRING ON THE BIOLOGICAL SENSOR SURFACE

The responsive processes that occur on the surface of a biological sensor include the diffusion process of basal substance and products, as shown in Fig. 2. After basal substance diffusion has entered the biological catalysis stage, the catalytic reaction produces the gas being observed; and finally, when the production rate of the product and the rate at which the product escapes the surface of the electrode through diffusion are equal, a stable

concentration is reached, and the corresponding stable electrical potential is measured. This potential and the basal substance concentration have a nengsite [transliteration] response.

Table 1. Varieties of Tissue Electrodes

Basic Substance	Biological Catalyst	Gas-sensitive Electrode
Cysteine	Cucumber leaf	NH_3 electrode [6]
Urea	Sword bean	NH_3 electrode [16]
Duoba [transliteration] amine	Banana flesh	O_2 electrode [9]
PO_4^{3-}/F^-	Potato stem tuber/glucose oxidizing enzyme	O_2 electrode [10]
Tyrosine	Beet	O_2 electrode [7]
Glutamic acid salts	Pumpkin	CO_2 electrodes [8]
Acetone acid salt	Corn kernel	CO_2 electrode [5]
Phenol varieties	Mushroom	O_2 electrode [4]
Adenosine	Mouse small intestine viscous membrane tissue	NH_3 electrode [14]
Adenosine 5'-monophosphate	Rabbit muscle	NH_3 electrode [13]
Adenosine 5'-monophosphate	Rabbit muscle ketone powder	NH_3 electrode [19]
Glutamine	Porcine kidney	NH_3 electrode [12]
Glutamine	Porcine kidney mitochondria	NH_3 electrode [22]
Bird purine	Rabbit liver	NH_3 electrode [15]
Glucose amine-6-phosphate	Porcine kidney	NH_3 electrode [17]
Superoxidized hydrogen	Cow liver	O_2 electrode [20][21]

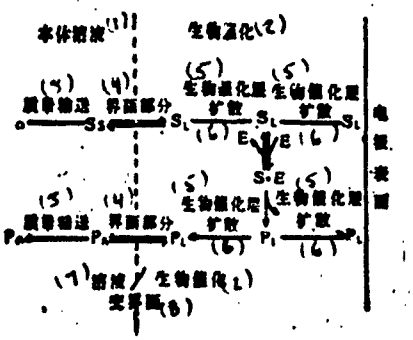

Fig. 2. Responsive process occurring on the surface of a biological sensor. Key: (S) Basal substance; (P) Product; (E) Enzyme; (1) Body solution; (2) Biological catalysis; (3) Substance? output; (4) Portion of boundary; (5) Biological catalysis layer; (6) Diffusion; (7) Solution; (8) Interchange surface.

AN EXAMPLE OF A TISSUE ELECTRODE

Of the plant tissue electrodes that have been reported to date, as shown in the first eight rows of Table 1, we shall choose one specific example. A thick syrup of sword bean is spread on the surface of an NH_3 electrode forming a urea-measuring electrode [16]. Making use of the large urea enzyme content of the sword bean, the decomposition of the urea into 1 mole of CO_2 and 2 moles of NH_3 is catalyzed. Measurement is performed with the NH_3 electrode. Figure 3 shows the regularized graph for an electrode made of sword bean; the buffer solution was 0.2 M Tris-HCl, with a pH of 8.5, within a 3.4×10^{-5} to 1.5×10^{-3} M linear range. The slope is 55.5 mv/dec to 58.5 mv/dec, and the limit of detection 1.2×10^{-6} M. The

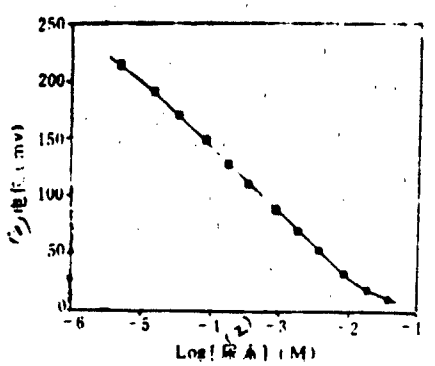

Fig. 3. Response graph of a urea electrode made with sword bean. Key: (1) Potential; (2) Urea.

response time in the linear range was 0.5-6 minutes, with a life of up to 94 days; experimentation was carried out on 19 kinds of substances that might generate interference, and none of them did generate interference.

The animal tissue electrodes that have been reported to date are shown in the last eight rows of Table 1. When a glutamine electrode [12] is formed by joining a porcine kidney skin layer with an NH_3 electrode, the catalytic response is as shown in Fig. 4, and the electrode response graph is as shown

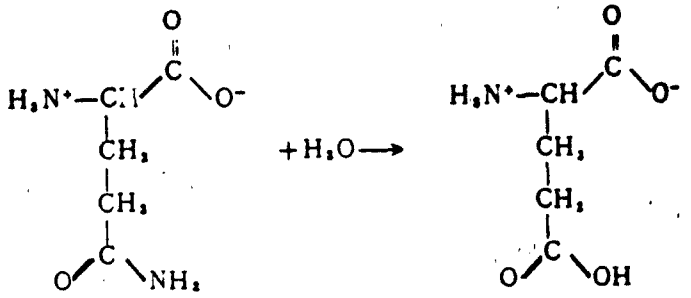

Fig. 4. Left: L-glutamine; Right: L-glutamic acid.

in Fig. 5. In a 0.1 M phosphate buffer solution with a pH of 7.8, containing 0.02% NaN_3 as a preservative and a response time of 5 to 10 minutes, the slope within the linear range was 53 mv/dec, and the life was at least 28 days. Measurement was undertaken for 13 kinds of substances that might cause interference, and there was no interference, showing that the selectivity of this electrode is very good.

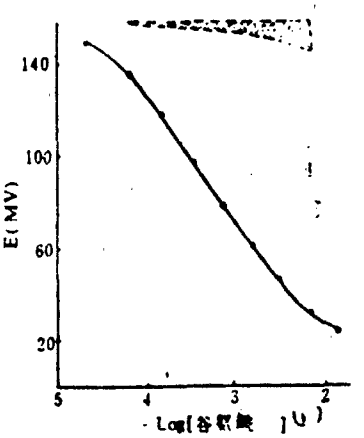

Fig. 4. Glutamine response graph and selectivity of porcine kidney tissue electrode. Key: (1) Glutamine.

In order to improve the measurement conditions of the tissue electrode to its maximum capability (that is, to raise the enzyme activity to its maximum amount), it is first necessary to select the pH value of the buffer solution. Figure 5 is

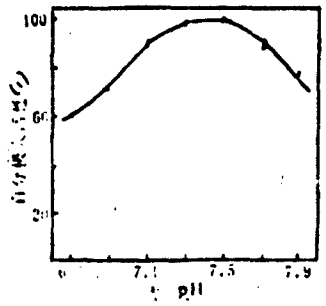

Fig. 5. Influence of pH on AMP rabbit muscle tissue electrode activity. Key: (1) 100 is the maximum activity.

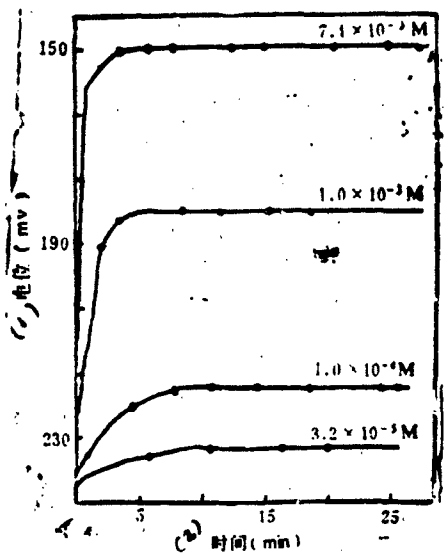

Fig. 6. Kinetic response of AMP tissue electrode at different levels of AMP concentration. Key: (1) Potential; (2) Time.

a sketch of the relationship between the pH of an adenosine 5'-monophosphate (AMP) rabbit muscle tissue membrane electrode [13] and the corresponding degree of activation. The ideal pH for the AMP tissue electrode is 7.5.

In order to obtain a certain constant potential, a certain time interval is necessary. It is necessary to make a stable state potential response time diagram. The stable state potential response time diagram for the AMP tissue electrode is shown in Fig. 6. From the figure, it can be seen that it takes at least 15 minutes to achieve a constant potential. This is basically in agreement with the results obtained by the author when using porcine kidney sections to make a glutamine measuring electrode.

The literature [15] reports that in making a tissue electrode it is necessary to add a preservative, to prevent the tissue section from being damaged by bacteria and shortening the lift of the electrode. In general, there are two kinds of preservatives. One is NaN_3; it is used in basic solutions with a concentration of 0.02%, but it will hinder the activity of several enzymes. The other kind of preservative is chlorine replacement 2-alkene-2-acetate; it can be used both in acid solutions and basic solutions with a concentration of 0.002%. The animal and plant tissue electrodes

reported in the literature, however, all use 0.02% NaN_3 as a preservative.

THE EFFECT OF GLYCERINE

In the literature on the L-glutamic acid tissue electrode [8], mention is made of the effect of glycerine. Glycerine and other solvents are able to stabilize the biological enzymes. When the glycerine concentration is 40%, the electrode activity is a fixed value; when the glycerine concentration is 30% or lower, the electrode activity increases or decreases according to the number of days. When there is no glycerine, the electrode's activity at the very beginning is very low; and when the glycerine concentration is greater than 50%, because of the viscosity of the glycerine, the electrode response time is too long. The author discovered, while making his porcine kidney glutamine electrode, that the electrode's activity was better after the tissue section was soaked in 40% glycerine than when it was not. This is in agreement with the reported effect of glycerine in protecting the cells and making the enzyme activity stable. But the question remains whether the presence of the glycerine changes the electrode's performance. In the literature [5] it is reported that 40% glycerine has no effect on the straight line range of CO_2 electrodes, the measurement limits or the response time.

INFLUENCE OF SOAKING ON ELECTRODE SENSITIVITY

Generally speaking, the sensitivity of tissue electrodes decreases as the number of days increases. After eight days, a tyrosine beet tissue electrode [7] shows no change in activity, but the degree of sensitivity of the electrode increases, as shown in Fig. 7. This is perhaps because of changes in tissue structure which give the biological catalytic activity layer even better permeability with respect to the base.

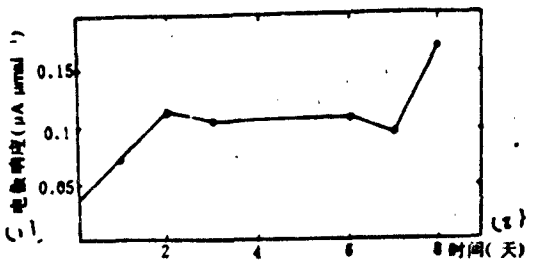

Fig. 7. Stability of a tyrosine electrode. Key: (1) Electrode response; (2) Time in days.

THE EFFECT ON THE ELECTRODE OF THE NYLON NET HOLE DIAMETER

The life of the electrode is affected by the specifications of the nylon net that is used in its construction. When using specifications of 149 μm, the biological catalytic activity diminishes rapidly; but with 37 μm there is a stabilizing influence. This is because damage to the tissue produces a lowering of activity.

ELECTRODE RECOVERY PERIOD AND EFFECT OF SECTION THICKNESS ON RESPONSE TIME

Electrode recovery period is defined as the time after measuring a given solution that the solution returns back to a neutral potential. The presence of the tissue section and the supporting membrane in general cause the recovery period of the tissue electrode to be slightly longer than the recovery time of the gas-sensitive electrode. When the AMP concentration goes from 2.5×10^{-4} to 9.1×10^{-3} M, the AMP tissue membrane electrode's recovery time goes from 19 minutes to 25 minutes; when the NH_4NO_3 concentration goes from 4.5×10^{-5} to 1.6×10^{-3} M, the NH_3 electrode's recovery time goes from 16 minutes to 18 minutes [13].

The effect of the section thickness on the response time is that the time increases as the tissue section thickens. The quantity of biological catalyst on the electrode surface increases, so the electrode's response time and recovery time increase. The lengthening of the time causes the advantages gained by the increase in the quantity of biological catalyst to lose significance. In actual practice, the maximum section thickness is 0.8 mm [15].

ELECTRODE LIFE, RENEWABILITY AND SELECTIVITY

The life, renewability and selectivity of the electrode are very important questions. If, for example, the other functions of a tissue electrode are very good but the renewability is deficient or the life is short, the electrode has little practical value. Figure 8 shows the adjusted

graph for the AMP tissue electrode for different lengths of days. It can be seen from the figure that this kind of electrode's response is renewable and reliable, and its minimum life is 28 days. When the tissue electrode exceeds this time period, the slope decreases owing to damage to the electrode and the response time increases. At this time the electrode is unable to continue in use. The life of tissue electrodes is generally calculated in days.

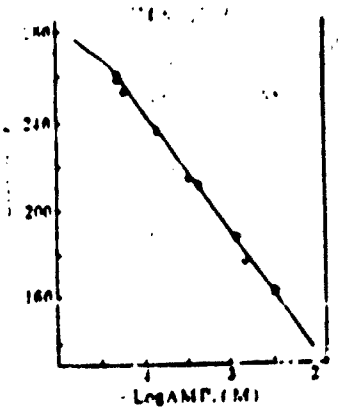

Fig. 8. Response of AMP tissue electrode. (○) is 2 days, (△) is 5 days, and (□) is 28 days.

If an electrode is responsive to a large number of substances, it has no practical value. Because many kinds of enzymes are contained in the tissue material, there are many metabolic styles, and it very important to be concerned with the tissue electrode's selectivity. The most important source of interference is other substances that are similar in structure to the substance to be measured and that produce electrode response under measurement conditions. Another source is that the reaction of the basal substance may change the electrode surface's pH value. The second kind of interference can be handled by increasing the base solution buffer content; but it is very difficult to eliminate or reduce the first kind of interference. We shall discuss two ways of overcoming it:

1) Selection of the ideal pH: Because the ideal pH value for the variety of enzymes in the tissue is not the same, it is desirable to raise the activity of the enzyme giving rise to the catalytic effect for the substance to be measured. It is therefore necessary to select the ideal pH value. In the pH - activity chart for the adenosine tissue electrode formed from rat small intestine viscous membrane cells matched with a NH_3 electrode, the ideal pH for adenosine is 9.0 to 9.4, while the ideal pH for AMP is 8.0. For the experiment, a pH of 9.0 was selected to overcome the interference of AMP.

2) Selection of an appropriate inhibitor: The most effective method for inhibiting interference is the method of ensuring the metabolism of the enzyme activity, and using a suitable inhibitor to inhibit the interference. By means of studying the process by which the adenosine tissue electrode is affected by possible interference from AMP, it has been discovered that AMP interference occurs in this way:

$$AMP + H_2O \xrightarrow[\text{(E.C.3.1.3.1.)}]{\text{Basic phosphate}} \text{Adenosine} + \text{phosphate}$$

$$\text{Adenosine} + H_2O \xrightarrow[\text{(E.C.3.5.4.4.)}]{\text{Adenosine deamination enzyme}} \text{Muscle glucoside} + NH_3$$

Further, on the basis of reports, the phosphate base ion blocks the activity of AMP deamination enzyme. Glycerine phosphate is also a blocking agent. Figure 9 shows the effect of using a adenosine tissue electrode to measure glycerine phosphate and phosphates on AMP and adenosine deamination enzyme. It can be seen from the figure that high concentration of phosphate causes the activity of AMP deamination to lower rapidly, while the adenosine deamination activity only decreases a very small amount.

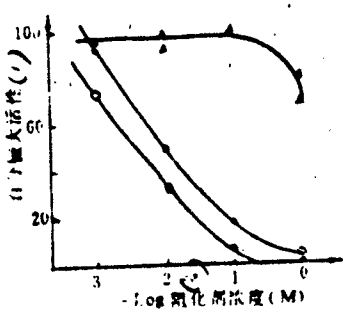

Fig. 9. Effect on AMP deamination enzyme (●, ○) and adenosine (▲, △) of glycerine phosphate and phosphates measured with a adenosine tissue electrode. Solid symbol, glycerine phosphate; open, phosphate. Key: (1) Max. act., 100; (2) Inhibitor concentration.

THE USE OF ACETONE POWDER

If it is difficult to obtain the tissue materials for constructing biological sensors, it is possible under these circumstances to use an acetone powder of the tissue material. An AMP electrode made using rabbit liver

acetone powder is very similar in function to an AMP electrode made using a rabbit liver tissue section. In comparison with enzyme electrodes, the life and slope of electrodes made in this manner are still very superior, and show little response to ADP [19].

CONSTRUCTING DIFFERENT TISSUE ELECTRODES FROM THE SAME BIOLOGICAL TISSUE

In porcine kidney there is both a large quantity of glucose amine-6-phosphate deamination isomer enzymes and glutamine hydrolyzed enzyme, but the pH value under which these two kinds of enzyme activities reach their maximum is not the same, the ideal value for the former being 9.25 and for the latter 8.7. When the pH is 9.25, the activity of the glutamine hydrolyzed enzyme is strongly inhibited; therefore it is possible to create a glucose amine-6-phosphate tissue electrode with having good selectivity and sensitivity and a long life.

COMPARISON OF RELATED BIOLOGICAL SENSORS AND TISSUE ELECTRODES

The widely accepted enzyme electrode has been challenged by sensors constructed from other biological catalytic material. These other materials include living bacteria cells and tissue sections; it is necessary to compare electrodes constructed from them (especially from tissue sections) with the enzyme electrode structure, performance and the sourcing and preservative methods for the material. From the comparison it will be possible to see that the tissue electrodes possess a number of advantages not found in the other electrodes. It can be seen from Table 2 that the tissue electrode has the longest life. This is because deamination enzyme in its natural surrounding of intact tissue is very stable. Within the range of experimental error, with the exception of the enzyme electrode, the slope, linear range, measurement limits and response time of the other three kinds of electrodes are comparable. The tissue electrode can be made and used under non-antiseptic conditions, while the bacterial electrode must be disinfected. The tissue of the tissue electrode is easy to preserve, while the price of enzymes for the enzyme electrode is high, and they must be stored at temperatures below

freezing. The tissue electrode's stability is higher than the enzyme electrode's, and it is simpler to construct. It can be made in a room-temperature buffer solution, while the enzyme electrode must be stored in freezing conditions to preserve its activity. It can be seen from these comparisons that the tissue electrode has incomparable advantages vis-à-vis the other biological electrodes. This is the reason for studying them.

Table 2. Response quality of four kinds of glutamine electrodes [3].

	(1)酶电极	(2)线粒体电极	(3)组织电极	(4)细菌电极
响应斜率(5)(mv/dec)	33.2—40.8	52.5±2.2	50.0±2.8	49
响应范围（平均）(6)	1.5×10^{-4}—3.3×10^{-3}M	1.1×10^{-4}—5.5×10^{-3}M	6.4×10^{-5}—5.2×10^{-3}M	1×10^{-4}—1×10^{-3}M
检测限（平均）(7)	6.0×10^{-5}M	2.2×10^{-5}M	2.0×10^{-5}M	5.6×10^{-5}M
响应时间(8)	4—5分(11)	6—7分(11)	5—7分(11)	5分(11)
寿命(9)	1天(12)	10天(12)	30天(12)	20天(12)
试验电极数(10)	3	3	11	8

Key: (1) Enzyme electrode; (2) Mitochondrial electrode; (3) Tissue electrode; (4) Bacteria electrode; (5) Response slope; (6) Response range, average; (7) Measurement limit, average; (8) Response time; (9) Live; (10) Number of experimental electrodes; (11) Minutes; (12) Days.

OTHER FACTORS TO BE CONSIDERED WHILE CONSTRUCTING A TISSUE ELECTRODE

1) The reversibility of the enzyme reaction must be small, the activation energy must be low, and the constant Km' must be as small as possible to guarantee the catalytic reaction's speed and completion. The data for the glutamine are as below; it fulfills these conditions:

$$\text{L-glutamine} + H_2O = \text{L-glutamate} + NH_3$$

$$K = \frac{(NH_4^+)(\text{L-Glutamate})}{(\text{L-glutamine})(H_2O)} = 320 \ (\text{pH}5.5,\ 25^\circ\text{C})$$

ΔG = 3400 Cal K'm = 5×10^{-3}

2) When selecting an enzyme source tissue, it is necessary to choose a tissue with a high enzyme activity with a high enzyme content. Porcine kidney tissue contains plentiful glutamine deamination enzyme.

3) As regards specialization of the enzyme, this factor determines the selectivity of the tissue electrode. If the specialization is low, there will necessarily be interference. Regarding porcine kidney glutamine tissue electrodes, there has been reported in the handbooks of enzymology and in the literature a total of 22 kinds of ammonia base acids that do not produce interference. It can be seen that the specialization of the enzyme is good.

4) It is necessary to consider the ideal pH for the enzyme catalytic reaction and the gas-sensitive electrode. The ideal pH conditions are when the ideal pH value for the enzyme reaction and the pH value required for the gas-sensitive electrode are as close as possible, in order to obtain satisfactory response characteristics. The ideal pH value for porcine kidney tissue electrodes measuring glutamine is 7.8, and the gas-sensitive electrode's optimum pH is 12; both are basic conditions.

APPLICATION

Using a tissue electrode constructed with porcine kidney to measure the concentration of glutamine in compound cerebral and spinal fluid [1], the quantity of glutamine added to the specimen both contained the normal concentration in human cerebral and spinal fluid and contained the concentration of glutamine of victims with liver coma or Reye syndrome. The glutamine concentration reported using the earlier method [23] was 2.6×10^{-3} M. Using the tissue electrode, it is measured as $2.4 \pm 0.1 \times 10^{-3}$M. The two results are quite close. Performing recovery rate experiments, the results are 98% to 104%. These results demonstrate that tissue electrodes have a very practical value.

Currently, tissue electrode technology is still in its earliest initial phase. It can be foreseen that it has a great future in the areas of biological medicine and clinical examination.

Professor Deng Mingtao and Assistant Professor Guo Dingli offered valuable advice for this project; we wish to express our great thanks.

REFERENCES

1. Arnold, M.A. and G.A. Rechnitz. Analytica Chimica Acta, 113, 351 (1980).

2. Ma Yilong. Guowai Fenxi Yiqi Jishu Yu Yingyong [Technology and Application of Foreign Analytic Instruments], 3-56 (1986).

3. Arnold, M.A. and G.A. Rechnitz. Anal. Chem. 52, 1170 (1980).

4. Lumir Macholàn. Lubomiv Schànei Biologia 39 (12), 1191 (1984).

5. Kuriyama, S., M.A. Arnold and G.A. Rechnitz. Journal of Membrane Science, 12, 26 (1983).

6. Smit, N., and G.A. Rechnitz. Biotechnology Letters, Vol. 6, No. 4, 209 (1984).

7. Shukert, F., U. Wollenberger, and F. Scheller. Biotechnology Letters, Vol. 5, No. 4, 239 (1983).

8. Kuriyama, S. and G.A. Rechnitz. Analytica Chimica Acta, 131, 91 (1984).

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